SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

Summary of Safety and Effectiveness Data

I. GENERAL INFORMATION

Device Generic Name:	Device for Detection of HER-2/*neu* Gene Amplification in Human Breast Tissue
Device Trade Name:	*HER2* FISH pharmDX™ kit
Applicant's Name and	DakoCytomation Denmark A/S
Address:	Produktionsvej 42 DK-2600 Glostrup Denmark
Premarket Approval Application (PMA) Number:	P040005
Date of Panel Recommendation:	None
Date of Notice of Approval to the Applicant:	May 3, 2005

II. INDICATIONS FOR USE

The DakoCytomation *HER2* FISH pharmDx™ Kit is a direct fluorescence *in situ* hybridization (FISH) assay designed to quantitatively determine the HER2 gene amplification in formalin-fixed, paraffin-embedded breast cancer tissue specimens. *HER2* FISH pharmDx™ Kit is indicated as an aid in the assessment of patients for whom Herceptin® (trastuzumab) treatment is being considered. Results from the *HER2* FISH pharmDx™ Kit are intended for use as an adjunct to the clinicopathologic information currently used for estimating prognosis in stage II, node positive breast cancer patients.

III. DEVICE DESCRIPTION

The *HER2* FISH pharmDx™ Kit is used for the identification and quantification of HER-2/*neu* gene amplification by fluorescent *in situ* hybridization on formalin-fixed, paraffin-embedded tissue sections fixed on slides. The kit contains the following principal component reagents: *HER2*/CEN-17 Probe Mix (Texas Red-Labeled *HER2* DNA probe and fluorescein-labeled CEN-17 peptide nucleic acid (PNA) probes in hybridization buffer with 45% formamide, stabilizer and unlabeled PNA blocking probes), ready-to use fluorescence mounting medium with DAPI (4,6 diamidino-2-phenylindole) counterstain, ready-to-use pepsin solution with stabilizer and antimicrobial agent, 20X Pretreatment Solution (MES buffer – 2-[N-

morpholino]ethanesulfuonic acid buffer), 20X Wash Buffer (Tris/HCl buffer) and coverslip sealant.

The *HER2*/CEN-17 Probe Mix consists of a mixture of Texas-Red labeled DNA cosmid clones that cover 200 kb of the chromosomal region that includes the *HER2* gene plus a mixture of fluorescein-labeled (PNA) probes targeted at the centromeric region of human chromosome 17. The probes are pre-mixed and pre-denatured in hybridization buffer for ease of use. Unlabeled blocking PNA probes are also included to suppress sequences contained within the target loci that are common to other chromosomes. Upon specific hybridization at the two targets, a red fluorescent signal is seen at each *HER2* gene and a green signal at the centromere. In each cell, the copy numbers of *HER2* and CEN-17 are enumerated. The presence of amplified *HER2* is determined by the ratio of the average copy number of *HER2* to CEN-17.

IV. CONTRAINDICATIONS

None known

V. WARNINGS AND PRECAUTIONS

Refer to the product labeling for a list of warnings and precautions.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Other commercially available FISH devices for gene amplification determination in breast tissue of lymph node negative patients with localized invasive tumor. Alternative procedures for detection of gene product overexpression in human breast tissue include immunohistochemical (IHC), or polymerase chain reaction (PCR) techniques.

VII. MARKETING HISTORY

The *HER2* FISH pharmDx™ Kit that is indicated for the detection of bladder cancer in symptomatic patients has not been marketed previously for clinical use.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON PUBLIC HEALTH

A potential risk associated with misuse of the assay, or a false positive test result is to assign patients to receive a more aggressive adjuvant therapy regimen than needed, possibly exposing the patient to serious side effects and, in rare cases, death. Alternatively, a false negative test result may exclude a patient who might benefit from more aggressive therapy from a treatment regimen, potentially resulting in a poor outcome.

IX. SUMMARY OF STUDIES

A. Non-Clinical Studies

1. Analytical

a) Hybridization Efficiency

Metaphase spreads from 3 control cell lines and 174 formalin-fixed, paraffin-embedded breast cancer tissue specimens were examined to identify chromosome 17 and the HER-2/*neu* gene locus by FISH. No cross-hybridization to other chromosome loci was observed in the cells examined. Hybridization was limited to the intended target regions of the two probes. The acceptance criterion specified that more than 95% of the hybridizations in both cell line and tissue sections had to be successful. The hybridization efficacy for hybridizations to control slides was 100% (43/43) and 96.6% for hybridizations to tissue sections (168/174).

b) Analytical Sensitivity

The analytical sensitivity of the *HER2* FISH pharmDx Kit probes was determined using two breast cancer cell lines, one without amplification (MDA-231) and one with amplification (SKBR3). The estimated mean ratio of *HER-2* and CEN-17 for MDA-231 was 1.12 (S.D. = 0.06) and for SKBR3, mean ratio was 3.65 (S.D. = 0.59).

c) Analytical Specificity

i. Locus Specificity

The *HER2* DNA probes in the *HER2*/CEN-17 Probe Mix have been end-sequenced and mapped to confirm a total coverage of 218 kb including the *HER2* gene. The CEN-17 PNA probes in the *HER2*/CEN-17 Probe Mix have been tested individually and in combination to confirm their specific hybridization to the centromeric region of chromosome 17.

To exclude cross-hybridization to chromosomes other than chromosome 17, metaphase spreads from normal lymphocytes were analyzed. A total of 250 metaphase spreads (5 lots with 50 metaphases in each lot) were examined sequentially by G-banding to identify chromosome 17 and the *HER-2* gene locus by FISH. All 250 metaphases had bright distinct signals located on chromosome 17. No cross-hybridization to other chromosome loci was observed; hybridization was limited to the intended target regions of the two probes.

ii. Stringency/Robustness Studies

Stringency studies were performed on formalin-fixed, paraffin-embedded tissue specimens to assess 1) optimum pre-treatment

conditions (at three different temperatures tested at three different times), 2) optimum Pepsin-treatment conditions (tested at five different durations), 3) hybridization time and temperature, and 4) post-hybridization time and temperature. Samples from mamma carcinoma tissue with and without amplification were used.

For the denaturation step, three temperatures (89°C, 92°C, and ≥95°C) were tested for 7, 10, and 13 minutes each. The results showed no significant difference in the overall score among all denaturation temperatures and durations. Based on the results from the study, pretreatment should be done for 10 minutes at ≥95°C.

Stringency of the hybridization step was tested in two parts; first, hybridizations were conducted at 3 different temperatures (40°C, 45°C, and 50°C) for 17 hours, then for 3 different durations (10 hr, 12 hr, and 14 hr) at the recommended temperature (45°C). None of the tested temperatures resulted in significant score deviations and scores lower than two were not seen. The recommended hybridization conditions are 14-20 hours at 45°C.

The post-hybridization wash step was tested at 3 different temperatures (60°C, 65°C, and 70°C) for 10 minutes, then for 3 different durations (5, 10, and 15 minutes) at 73°C. Wash temperature was a significant factor, with unacceptable signal intensities and score deviations higher than the allowed -1/2 were seen with 70°C. Wash times between 5 and 10 minutes produced acceptable results. Based on these results, the recommended post-hybridization wash conditions are 65°C for 10 minutes.

The effect of changing salt and detergent concentration in the wash buffer used in the post-hybridization step on staining results was also analyzed. Five different salt concentrations were made by diluting the 20x concentrated Stringent Wash Buffer from 1:10 to 1:40 with 1:20 being the recommended dilution. Two breast carcinoma tissue sections, one with HER-2 gene amplification and the other without were used. Results showed dilutions 1:10, 1:15, 1:20 and 1:30 met the acceptance criteria whereas the dilution 1:40 failed because of low signal intensity of the red HER-2 signals. A dilution of 1:10 to 1:30 corresponds to salt concentrations of 0.66 to 0.22 nmol/L. The assay can tolerate dilutions from 1:10 to 1:30.

d) Stability

For product expiration dating, the applicant performed real-time stability studies at -18°C and 2-8 °C for up to 25 months. The stability of the buffer at 2-8 °C and the effect of 15 freeze-thaw cycles were also assessed. In a separate study, the applicant evaluated the stability of the kits, i.e., 13 months at -18°C and 15 freeze

thaw cycles, and 12 months storage at 2-8 °C. The performance of the kits was measured on tissue specimens according to the device labeling. The data show consistent results under the storage conditions tested for up to 25 months.

The protocol used to establish this expiration dating is considered an approved protocol for the purpose of extending the expiration dating as provided by 21 CFR 814.39(a)(8).

For specimen stability, cut sections from four formalin-fixed, paraffin embedded breast carcinoma tissue blocks (two had *HER2* gene amplification and two non-amplified) stored at 2-8°C were tested over a period of 31 months. The cut sections were evaluated for signal intensity of the *HER2* and the CEN 17 probe as well as tissue morphology. For each section, 20 nuclei were counted. The data show acceptable consistent results under the storage conditions tested for up to 31 months or 7.3 months at 25°C.

2. Repeatability Study

Repeatability of the *HER2*/CEN-17 ratio was determined using consecutive sections of normal breast tissue and breast carcinoma tissue. The coefficient of variation for normal breast tissue was found to be 6% and for breast carcinoma 4%. The effect of different tissue thickness was also investigated. A total of 10 consecutive sections of breast cancer tissue with different thickness (duplicates of 3, 4, 5, 6 and 7 µm) were tested. The coefficient of variation of the *HER2*/CEN-17 ratio was found to be 12% which was higher than for tissue sections of equal thickness.

3. Reproducibility Studies

a) Breast Cancer Specimens

i. Site-to-Site Reproducibility (Portability)

A five-center, blinded, randomized, comparative study using 4 neutral buffered formalin-fixed, paraffin-embedded human breast cancer specimens with different levels of *HER-2* gene amplification (two non-amplified, one low level and one high level) with was conducted to assess assay portability. Each laboratory designated one technician and one pathologist for the staining and evaluation respectively. 60 nuclei were counted for each specimen. Each specimen was counted on 3 different days. Site-to-site variation for the *HER2*/CEN-17 ratio of 10-15% was observed for the non-amplified cases and cases with ratios close to the cut-off. For highly amplified specimen, variation observed was 25% because of clustering of signals which made accurate counting difficult.

ii. Day-to-Day Reproducibility

A day-to-day variation of 10% was found based on data from the site-to-site study.

iii. Observer-to-Observer Reproducibility

For this study, 32 different breast cancer specimens with different levels of HER-2 gene amplification (*HER-2*/CEN-17 ratios ranged from 0.9 to 13.0) were selected and tested with the *HER2* FISH kit. These samples were from archived specimens at DakoCytomation and had previous *HER2* assessment either by HercepTest™ or by FISH analysis. Twenty nuclei were counted for each specimen. Each specimen was counted by 3 observers. Twenty-seven of the 32 specimens had evaluable results. Of the 27 samples, 20 were non-amplified and 7 amplified. Variation in scoring between observers was not significant for negative specimens but higher variability was observed for amplified specimens. Results showed 100% concordance between the observers. Reliability of ratings was assessed by the Shrout-Fleiss single score reliability method. The Shrout Fleiss single score reliability was 0.94405 indicating strong agreement among observers.

b) Cell Lines

i. Lot-to-Lot Reproducibility

The kit was tested for lot-to-lot reproducibility of three lots of test kits using 3 different formalin-fixed paraffin-embedded cell lines (MDA-231, MDA-175 and SKBR3). Five slides of each cell line were tested in one day. Thirty nuclei were counted.

Cell line	HER2/CEN-17 Ratio	Lot 1	Lot 2	Lot3	Total
MDA-231	Mean	1.06	1.04	1.07	1.06
	SD	0.04	0.04	0.05	0.04
	CV%	3.7	3.8	4.7	3.8
	Number of slides	5	5	5	15
MDA-175	Mean	1.23	1.20	1.16	1.20
	SD	0.02	0.05	0.07	0.06
	CV%	1.6	4.2	6.0	5.0
	Number of slides	5	5	5	15
SKBR3	Mean	3.99	3.77	3.82	3.86
	SD	0.18	0.19	0.29	0.23
	CV%	4.5	5.0	7.6	6.0
	Number of slides	5	5	5	15

ii. Day-to-Day Reproducibility

The kit was tested for day-to-day reproducibility over 4 days 2 months apart using 3 different formalin-fixed paraffin-embedded cell lines (MDA-231, MDA-175 and SKBR3). Five slides of each cell line were tested each day. Thirty nuclei were counted.

Cell Line	HER2/CEN-17 Ratio	Day 1	Day 2	Day 3	Day 4	Total
MDA-231	Mean	1.04	1.03	1.05	0.99	1.03
	SD	0.05	0.02	0.03	0.01	0.04
	CV%	4.8	1.9	2.9	1.0	3.9
	Number of slides	5	5	5	5	20
MDA-175	Mean	1.26	1.17	1.25	1.16	1.21
	SD	0.06	0.04	0.07	0.04	0.07
	CV%	4.8	3.4	5.6	3.4	5.8
	Number of slides	5	5	5	5	20
SKBR3	Mean	4.30	4.59	4.56	4.09	4.39
	SD	0.39	0.32	0.15	0.08	0.32
	CV%	9.1	7.0	3.3	2.0	7.3
	Number of slides	5	5	5	5	20

iii. Observer-to-Observer Reproducibility

The kit was tested for observer-to-observer reproducibility over 3 observers using 3 different formalin-fixed paraffin-embedded cell lines (MDA-231, MDA-175 and SKBR3). Five slides of each cell line were tested per observer. Thirty nuclei were counted.

Cell Line	HER2/CEN-17 Ratio	Obs 1	Obs 2	Obs 3	Total
MDA-231	Mean	1.03	1.03	1.09	1.05
	SD	0.02	0.08	0.05	0.06
	CV%	1.9	7.8	4.6	5.7
	Number of slides	5	5	5	15
MDA-175	Mean	1.17	1.15	1.11	1.14
	SD	0.02	0.05	0.10	0.07
	CV%	1.7	4.3	9.0	6.1
	Number of slides	5	5	5	15
SKBR3	Mean	4.03	3.57	3.63	3.74
	SD	0.18	0.19	0.24	0.29
	CV%	4.5	5.3	6.6	7.8
	Number of slides	5	5	5	15

3. Normal Range Determination

Twenty-one normal breast tissue as defined by H&E staining were tested with the *HER2* FISH pharmDx™ Kit. Ratios of *HER2*/CEN-17 were calculated based on 20 and 60 counted nuclei. The mean ratio for the 20 nuclei was 1.06

with a 95% confidence interval (CI) of 1.02-1.10. The minimum observed ration was 0.86 and the maximum ratio was 1.19. The standard deviation was 0.09. Counting 60 nuclei did not significantly change the results. The mean ratio was 1.06 with a 95% CI of 1.03-1.08. The minimum ratio was 0.94 and the maximum ratio was 1.16. The standard deviation was 0.06.

4. Alternative Counting Method

The applicant proposed an alternative counting method which counts a fixed number of fluorescent signals or events instead of a fixed number of nuclei/cells as in the conventional method. For the alternative method, the number of nuclei to be counted will depend on the strength and number of signals and will count more nuclei in cases near the cut-off than in highly amplified nuclei. Simulation studies were performed on data with complete 60 cell evaluation by both *HER2* FISH pharmDx™ Kit and PathVysion Her2 DNA Probe Kit by counting 10, 20, 30, and 60 events, as well as 10, 20, and 30 nuclei. Statistical analysis showed a total agreement of 0.94 when as few as 20 events (minimum of 7 nuclei) were counted with 90% of the cases, ≤15 actual nuclei counted. When 30 or 60 events (minimum of 7 nuclei) were counted, the total agreement was 0.97 and the actual number of nuclei counted was ≤20 in 90% of the cases.

B. Clinical Validation

Three studies have been performed to compare results of the *HER2* FISH pharmDx™ Kit to both the HercepTest™ and the PathVysion™ HER-2 DNA Probe Kit. The primary study was the Danish study which is described below.

1. Danish Study

For this study, the selection, staining and interpretation of the stained slides using the *HER2* FISH pharmDx™ kit was conducted at the Department of Pathology at Roskilde Hospital in Denmark. The PathVysion™ HER-2 DNA Probe test was conducted at Laboratory Corporation of America (LabCorp), Research Triangle Park, North Carolina, USA with no prior knowledge of the data generated by the DakoCytomation test.

The study specimens were selected from archived patient breast tumor tissue specimens collected for the Danish Breast Cancer Group (DBCG) clinical trial 89-D in 1990. The objective of the trial was to test the effectiveness of CMF (cyclophosphamide, methotrexate and 5-FU) or CEF (cyclophosphamide, epirubicin and 5-FU) therapy for patients with a diagnosis of Stage II or Stage III breast cancer. The trial enrolled 980 Danish cases and 906 specimens were available for testing. No study outcome information was made available to the applicant. The following table summarizes patient demographics.

<u>Subject demographics</u>

The following table summarizes the demographics of the 980 enrolled subjects.

Variable	Mean	SD	Minimum	Maximum
Age (y)	47.68	9.03	24	69
Menopause status	Pre = 284; Post = 696			
# positive nodes	3.15	4.47	0	30
Tumor size (cm)			1	121

Of the 980 samples, 820 had valid HercepTest™ results. The distribution shows a higher than average percentage of 3+ cases, due primarily to the disease status of the enrolled subjects. Of the 820 samples, 682 were tested with the *HER2* FISH pharmDx™ Kit. The distribution of HER2 status by HercepTest™ and *HER2* FISH pharmDx™ is presented below.

HercepTest Score	0	1	2	3	Total
N	221	267	84	248	820
%	26.95%	32.56%	10.24%	30.24%	100%
HER2 FISH status					682
Non-amplified	106	245	65	22	435
Amplified	0	8	17	222	247

a) Comparison Study with HercepTest™

Correlation of *HER2* FISH pharmDx™ test results to HercepTest™

	HercepTest™ result		
HER2 FISH pharmDx™	Positive (≥3+)	Negative(<3+)	Total
Amplified	222	25	247
Non-amplified	22	413	435
Total	244	438	682

Positive percent agreement = 222/244 = 0.9098 (95% CI: 0.8739, 0.9457)
Negative percent agreement = 413/438 = 0.9429 (95% CI: 0.9212, 0.9646)
Total percent agreement = 635/682 = 0.9311 (95% CI 0.9121, 0.9501)

Using 3+ by HercepTest™, the results showed a 2x2 concordance of 93% (95% CI 91% - 95%).

b) Comparison Study with the PathVysion™ HER-2 DNA Probe Kit

Initially for *HER2* FISH and PathVysion Her-2 DNA Probe testing, 150 specimens were sampled from the 645 samples by proportional stratified systematic sampling. Stratification was based on the scoring results of the HercepTest™ and to reflect the distribution in the 645 samples with representative percentage for each staining intensity. For each staining intensity, every 4th patient specimen was chosen. If there were tissue issues when evaluated by H&E staining, the tissue specimen was not included in subsequent testing and another from that staining intensity was selected. If the tissue failed the *HER2* FISH test, that specimen was replaced with

another specimen in the study. The following table shows the distribution of the samples according to HercepTest™ results.

	Original sample set		Selected for *HER2* FISH	
	# patients	Percent	# patients	Percent
HercepTest 0	169	26.2	39	26
HercepTest 1+	210	32.56	49	32.67
HercepTest 2+	65	10.08	15	10
HercepTest 3+	201	31.16	47	31.33
# patients tested	645	100	150	100
Invalid	261			
Total	906		150	

Since the original data did not have sufficient HercepTest™ 2+ tumor samples, the remaining 50 HercepTest™ 2+ samples from the DBCG 89-D study were added. Thus the total number of tumor samples for the comparison study was 200.

For each tumor tissue, 12 serial sections were made and each section was assigned to be used for a specific test. Testing and scoring of FISH signals were performed according to the manufacturers' instructions. Sixty nuclei were counted for both *HER2* FISH and PathVysion HER2 tests for the 150 samples in the original set and for the additional 50 HercepTest™ 2+ samples, a minimum 60 *HER2* events per specimen were counted.

Staining with the Vysis PathVysion™ HER-2 DNA Probe was successful for 145 of the 150 samples in the original set and 50 of 50 in the second set. Of the 195 samples, 190 were evaluable for both *HER2* FISH pharmDx™ and PathVysion ™ HER-2 DNA Probe tests.

The following table shows distribution of the PathVysion HER-2 DNA Probe and the *HER2* FISH pharmDx™ results in relation to the HercepTest™.

HercepTest Score	0	1	2	3	Total
N	221	267	84	248	820
%	26.95%	32.56%	10.24%	30.24%	100%
PathVysion status					195
Non-amplified	37	45	50	4	136
Amplified	0	2	15	42	59
PathVysion result not available	2	1	1	1	5
HER2 FISH results					190
Non-amplified	37	45	49	5	136
Amplified	0	2	11	41	54
HER2 FISH result no available	0	0	5	0	5

Correlation of *HER2* FISH pharmDx™ test results to HercepTest™ for the 190 samples used in the comparison study with PathVysion™ HER-2 DNA Probe test

HER2 FISH pharmDx™	HercepTest™ result	

	Positive (≥3+)	Negative(<3+)	Total
Amplified	40	14	54
Non-amplified	6	130	136
Total	46	144	190

Positive percent agreement = 40/46 = 0.8696 (95% CI: 0.7726, 0.9669)
Negative percent agreement = 130/144 = 0.9028 (95% CI: 0.8544, 0.9512)
Total percent agreement = 170/190 = 0.8947 (95% CI: 0.8511, 0.9383)

Using 3+ by *HercepTest*, the results showed a 2x2 concordance of 89.47% (95% CI 85.11% - 93.83%). The Kappa statistics was 0.7292, with a 95% CI of 0.6185-0.8399 indicating that the *HER2* FISH pharmDx™ test is substantially equivalent to HercepTest™.

Correlation of PathVysion™ HER2 Probe test results to HercepTest™ for the 190 samples used in comparison study with *HER2* FISH pharmDx™ test

	HercepTest result		
PathVysion™ HER2 Probe results	Positive (≥3+)	Negative(<3+)	Total
Amplified	42	16	58
Non-amplified	4	128	132
Total	46	144	190

Positive percent agreement = 42/46 = 0.9130 (95% CI: 0.8316, 0.9944)
Negative percent agreement = 128/144 = 0.8889 (95% CI: 0.8376, 0.9402)
Total percent agreement = 170/190 = 0.8947 (95% CI: 0.8511, 0.9383)

Using 3+ by *HercepTest*, the results showed a 2x2 concordance of 89.47% (95% CI 85.11% - 93.83%). The Kappa statistics was 0.7366, with a 95% CI of 0.6293-0.8438.

Correlation of PathVysion™ HER-2 Probe test to *HER2* FISH pharmDx™ test using *HER2*/CEN-17 ratio of 2.0 as the cut-off value

	PathVysion™ HER-2 Probe result		
HER2 FISH pharmDx™	Amplified	Non-amplified	Total
Amplified	50	4	54
Non-amplified	8	128	136
Total	58	132	190

Positive percent agreement = 50/58 = 0.8621 (95% CI: 0.7734, 0.9508)
Negative percent agreement = 128/132 = 0.9697 (95% CI: 0.9405, 0.9989)
Total percent agreement = 178/190 = 0.9368 (95% CI: 0.9022, 0.9714)

The following table summarizes the 12 discrepant test results between *HER2* FISH pharmDx™ test and PathVysion™ HER-2 Probe test

HER2 FISH(+)/PathVysion(-)				*HER2* FISH(-)/PathVysion(+)			
ID	*HER2* FISH	PathVysion	HercepTest	ID	*HER2* FISH	PathVysion	HercepTest
160	2.10*	1.51	2	234	1.68	2.02*	2

	(1.82-2.51)	(1.39-1.68)			(1.38-1.83)	(1.84-2.29)	
208	3.61 (2.95-4.73)	1.62 (1.51-1.82)	2	284	1.44 (1.07-1.83)	2.21* (1.94-2.64)	2
306	2.20* (1.79-2.24)	1.33 (1.18-1.44)	1	423	1.7 (1.52-1.95)5	2.15 (2.02-2.45)	1
846	2.58 (2.06-3.50)	1.51 (1.42-1.76)	2	474	1.44 (1.16-1.83)	2.55 (2.38-3.26)	2
				735	1.68 (1.40-1.99)	2.03* (1.89-2.19)	2
				746	1.05 (0.96-1.18)	4.53 (4.27-5.17)	3
				837	1.52 (1.48-1.79)	2.15 (2.10-2.67)	3
				881	1.83* (1.15-2.69)	2.68 (2.39-3.14)	2

*CI of mean log ratios included 2.0

In this discrepancy analysis, logged ratios were used. The 95% confidence interval was calculated for the 60 logged ratios from the nuclei that were used to calculate the PathVysion ratio. For the 4 instances where *HER2* FISH was positive and PathVysion was negative, no interval included the critical value of 2. Of the eight instances where *HER2* FISH was negative and PathVysion was positive, the 95% CI of 3 (#234, 284 and 735) included the critical value of 2.

Similarly, The 95% confidence interval was calculated for the logged ratios from the nuclei that were used to calculate the *HER2* FISH ratio. For the 4 instances where *HER2* FISH was positive and PathVysion was negative, 2 included the critical value of 2 (#160 and 306). Of the eight instances where *HER2* FISH was negative and PathVysion was positive, the 95% CI of 1 (#881) included the critical value of 2.

2. Additional Studies

Testing was performed using the *HER2* FISH pharmDx™ Kit and the PathVysion™ HER-2 DNA Probe Kit on 52 samples in Japan. The specimens were randomly collected from Denmark, USA and Japan and with HercepTest results. The specimens included 17 HercepTest 0, 6 specimens at 1+, 5 specimens at 2+ and 24 specimens 3+ (see table below)

HercepTest Result	0	1+	2+	3+
Denmark	10	2	3	9
USA	0	0	0	5
Japan	7	4	2	10
Total	17	6	5	24

One technician performed the staining and two technicians for scoring. Sixty nuclei per specimen were counted. Results are summarized below.

HER2 FISH pharmDx™	PathVysion™ HER-2 Probe result	

	Amplified	Non-amplified	Total
Amplified	28	1	29
Non-amplified	1	22	23
Total	29	23	52

Positive percent agreement = 28/29 = 0.9655
Negative percent agreement = 22/23 = 0.9565
Total percent agreement = 50/52 = 0.9615

Discrepant samples are shown below.

Specimen no.	HER2/CEN-17 ratio				IHC
	HER2 FISH		PathVysion		
12	1.8	Non-amp	2.7	Amp	3+
26	2.4	Amp	1.9	Non-amp	3+

Testing was also performed using the *HER2* FISH pharmDx™ Kit and the PathVysion™ HER-2 DNA Probe Kit on 86 samples in France. The specimens were from Institute Paoli-Calmettes. Thirty-four samples were amplified and 52 were not. No differences were observed between the two assays.

3. Combined Study Results

Study Designation	Danish	Japanese	French
Number of samples	190	52	86
Positive percent agreement	86%	97%	100%
Negative percent agreement	97%	96%	100%
Total percent agreement	94%	96%	100%

X. CONCLUSIONS DRAWN FROM THE STUDIES

It is believed that the previous studies demonstrate the following:

- Acceptable performance is obtained with the *HER2* FISH pharmDx™ device on formalin-fixed, paraffin-embedded human breast cancer tissue sections with varying levels of gene amplification.

- The *HER2* FISH pharmDx™ device demonstrated inter-day, inter-lot and inter-site reproducibility of <15% CV for normal and weakly amplified specimens and <25% CV for moderately and highly amplified specimens. The variability between observers reflects subjectivity in signal interpretation and enumeration and can be minimized by adequate training and proficiency assessment prior to test implementation. The overall hybridization success rate is 98%.

- The limit of detection as defined by the *HER2* to CEP 17 ratio for the *HER2* FISH pharmDx™ device is estimated to be 1.5. The estimated mean ratio for

5 non-amplified tissue specimens with a *HER2* to CEP 17 ratio of 0.98 to 1.15 was 1.05±0.07.

- Comparable amplification results can be obtained by enumerating 20 nuclei instead of 60 nuclei as originally recommended. Additional nuclei should be counted for results at or near the cutoff point (1.8 to 2.2) or if there is significant variability in signal number from nucleus to nucleus.

- The *HER2* FISH pharmDx™ Kit can be stored up to 25 months at 2-8 °C.

- Based on clinical laboratory studies, the *HER2* FISH pharmDx™ Kit when used in accordance with the provided directions and in conjunction with clinical information, is safe and effective in the determination of the *HER2* gene amplification status in patients with stage II, node-positive breast cancer. Concordance with immunohistochemistry (IHC) was found to be 93% (95% CI: 91%-95%). In addition, *HER2* FISH pharmDx™ Kit results are comparable to the PathVysion™ HER-2 DNA Probe assay which was approved for the same indication. The percent of total agreement was 94% (95% CI: 0.90%-97%)

Safety

As a diagnostic test, the *HER2* FISH pharmDx™ Kit involves testing on formalin-fixed, paraffin embedded human breast cancer tissue sections. These tissue sections are routinely removed for breast cancer diagnosis. The test, therefore, presents no additional safety hazard to the patient being tested.

Benefit/Risk

The submitted clinical studies have shown that the *HER2* FISH pharmDx™ Kit, when compared to the reference method IHC, has similar ability to detect *HER2* amplification in specimens from patients with stage II, node positive breast cancer. The rate of false positivity and false negativity are within acceptable limits compared to the reference methods. Thus, this device should benefit the physician in assessing patients for HERCEPTIN (Trastuzumab) treatment and patients treated with adjuvant CAF chemotherapy.

Based on the results of the preclinical and clinical studies, the *HER2* FISH pharmDx™ Kit, when used according to the provided directions and in conjunction with clinical information, should be safe and effective and pose minimal risk to patient due to false test results.

XI. CDRH DECISION

FDA issued an approval order on May 3, 2005.

The applicant's manufacturing and control facilities were inspected on May 13, 2004 and the facilities were found to be in compliance with the Quality System Regulation (21 CFR 820).

XII. APPROVAL SPECIFICATIONS

Directions for use: See labeling

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions and Adverse Events in the labeling.

Postapproval Requirements and Restrictions: See approval order.